

-continued

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 duplex, antisense
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 Figure 18

 <400> SEQUENCE: 96
 ucgaaguaau ccgcguacgt t

21

The invention claimed is:

1. A method of preparing a double-stranded RNA molecule, wherein each RNA strand has a length from 19–25 nucleotides, wherein said RNA molecule is capable of target-specific nucleic acid modifications and wherein at least one strand has a 3'-overhang of 1–5 nucleotides, comprising

- (a) synthesizing two RNA strands each having a length from 19–25 nucleotides, wherein said RNA strands are capable of forming a double-stranded RNA molecule,
- (b) combining the synthesized RNA strands under conditions, wherein a double-stranded RNA molecule which mediates target-specific nucleic acid modifications is formed, wherein said double-stranded RNA molecule consists of a single double stranded region and single stranded regions of 1 to 5 nucleotides at the 3' ends of at least one of the strands of said double-stranded RNA molecule.

2. The method according to claim 1, wherein the RNA strands are chemically synthesized.

3. The method according to claim 1, wherein the RNA strands are enzymatically synthesized.

4. The method of claim 1, wherein both strands of said double-stranded RNA each have a 3'-overhang from 1–5 nucleotides.

5. The method of claim 1, wherein both strands of said double-stranded RNA each have a 3'-overhang from 1–3 nucleotides.

6. The method of claim 1, wherein both strands of said double-stranded RNA each have a 3'-overhang of 2 nucleotides.

7. The method of claim 1, wherein each strand has a length from 20–22 nucleotides.

8. The method of claim 7, wherein both strands of said double-stranded RNA each have a 3'-overhang from 1–5 nucleotides.

9. The method of claim 7, wherein both strands of said double-stranded RNA each have a 3'-overhang from 1–3 nucleotides.

10. The method of claim 7, wherein both strands of said double-stranded RNA each have a 3'-overhang of 2 nucleotides.

11. The method of claim 1, wherein the double-stranded RNA comprises at least one sugar-modified ribonucleotide, wherein the 2'-OH group of said sugar-modified ribonucleotide is replaced by a group selected from H, OR, R, halo, SH, SR, NH₂, NHR, N(R)₂ or CN, wherein R is C₁–C₆ alkyl, alkenyl or alkynyl and halo is F, Cl, Br or I.

12. The method of claim 1, wherein the double stranded RNA comprises at least one backbone-modified ribonucleotide containing a phosphorothioate group.

13. A method of preparing a double-stranded RNA molecule, wherein each strand has a length of from 19–25 nucleotides, wherein said RNA molecule is capable of mediating the cleavage of a target mRNA in a mammal and at least one strand has a 3' overhang of 1–3 nucleotides, comprising the steps of:

- a) selecting a target mammalian mRNA or target gene sequence,
- (b) synthesizing a first RNA strand having a length from 19–25 nucleotides, wherein said first RNA strand is complementary to contiguous nucleotides in said target mammalian mRNA or said target gene sequence,
- (c) synthesizing a second RNA strand having a length from 19–25 nucleotides, wherein said second RNA strand is complementary to 16–24 nucleotides from said first RNA strand, and
- (d) combining the synthesized RNA strands under conditions suitable to form a double stranded RNA molecule, wherein said double stranded RNA molecule consists of a single double stranded region of from 16–24 nucleotides in length and one or two single stranded 3' overhang regions of 1–3 nucleotides in length each.

14. The method of claim 13, wherein both strands of said double-stranded RNA each have a 3'-overhang from 1–3 nucleotides.

15. The method of claim 13, wherein both strands of said double-stranded RNA each have a 3'-overhang of 2 nucleotides.

16. The method of claim 13, wherein each strand has a length from 20–22 nucleotides.

17. The method of claim 16, wherein both strands of said double-stranded RNA each have a 3'-overhang from 1–3 nucleotides.

18. The method of claim 16, wherein both strands of said double-stranded RNA each have a 3'-overhang of 2 nucleotides.

19. The method of claim 13, wherein the double-stranded RNA comprises at least one sugar-modified ribonucleotide, wherein the 2'-OH group of said sugar-modified ribonucleotide is replaced by a group selected from H, OR, R, halo, SH, SR, NH₂, NHR, N(R)₂ or CN, wherein R is C₁–C₆ alkyl, alkenyl or alkynyl and halo is F, Cl, Br or I.

20. The method of claim 13, wherein the double stranded RNA comprises at least one backbone-modified ribonucleotide containing a phosphorothioate group.